

LONDON  
SCHOOL of  
HYGIENE  
& TROPICAL  
MEDICINE



Pinto, E; Barros, H; dos Santos Silva, I (2008) Dietary intake and nutritional adequacy prior to conception and during pregnancy: a follow-up study in the north of Portugal. *Public health nutrition*, 12 (7). pp. 922-31. ISSN 1368-9800 DOI: 10.1017/S1368980008003595

Downloaded from: <http://researchonline.lshtm.ac.uk/5413/>

DOI: [10.1017/S1368980008003595](https://doi.org/10.1017/S1368980008003595)

#### Usage Guidelines

Please refer to usage guidelines at <http://researchonline.lshtm.ac.uk/policies.html> or alternatively contact [researchonline@lshtm.ac.uk](mailto:researchonline@lshtm.ac.uk).

Available under license: <http://creativecommons.org/licenses/by-nc-nd/2.5/>

# Dietary intake and nutritional adequacy prior to conception and during pregnancy: a follow-up study in the north of Portugal

Elisabete Pinto<sup>1,\*</sup>, Henrique Barros<sup>1</sup> and Isabel dos Santos Silva<sup>1,2</sup>

<sup>1</sup>Department of Hygiene and Epidemiology, University of Porto Medical School, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal; <sup>2</sup>Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

Submitted 29 January 2008; Accepted 16 July 2008; First published online 27 August 2008

## Abstract

**Objective:** To assess maternal diet and nutritional adequacy prior to conception and during pregnancy.

**Design:** Follow-up of a cohort of pregnant women with collection of questionnaire data throughout pregnancy and after delivery.

**Setting:** Antenatal clinics at two public hospitals in Porto, Portugal.

**Subjects:** Two hundred and forty-nine pregnant women who reported a gestational age below 13 weeks at the time they attended their first antenatal visit.

**Results:** Intakes of energy and macronutrients were within recommended levels for most women. Pregnancy was accompanied by increases in the dietary intake of vitamins A and E, riboflavin, folate, Ca and Mg, but declines in the intake of alcohol and caffeine. The micronutrients with higher inadequacy prevalences prior to pregnancy were vitamin E (83%), folate (58%) and Mg (19%). These three micronutrients, together with Fe, were also those with the highest inadequacy prevalences during pregnancy (91%, 88%, 73% and 21%, respectively, for folate, Fe, vitamin E and Mg). Ninety-seven per cent of the women reported taking supplements of folic acid during the first trimester, but the median gestational age at initiation was 6.5 (interquartile range 5, 9) weeks. Self-reported prevalences of Fe and Mg supplementation were high, and increased throughout pregnancy.

**Conclusion:** The study identified low dietary intakes of vitamin E, folate and Mg both in the preconceptional period and during pregnancy, and low intake of Fe during pregnancy only. The low dietary intake of folate and the late initiation of supplementation indicate that current national guidelines are unlikely to be effective in preventing neural tube defects.

## Keywords

Diet  
Maternal nutrition  
Preconception care  
Prenatal care  
Dietary supplementation

Maternal nutrition during pregnancy has been linked to birth outcomes<sup>(1–7)</sup> (e.g. fetal growth<sup>(1,3,8)</sup>, gestation length<sup>(7,9)</sup> and birth defects<sup>(10)</sup>) and long-term health of the offspring through intra-uterine programming in early fetal life and its impact on the susceptibility to CVD and type 2 diabetes mellitus<sup>(11)</sup>. A woman's diet during pregnancy has also been associated with her risk of developing hypertensive disorders during pregnancy<sup>(4,12)</sup> and obesity later in life<sup>(13)</sup>.

Less is known about the impact of diet prior to and around conception on pregnancy outcomes as research has focused mainly on diet during mid to late pregnancy. Animal studies suggest that nutrition may influence oocyte and embryo quality, and thereby the proficiency of the fetus, fetal development and adult health outcomes<sup>(3)</sup>. In human subjects, pre-pregnancy maternal obesity, a broad

indicator of energy intake prior to conception, is associated with size at birth and adverse pregnancy outcomes<sup>(14)</sup>. Intake of micronutrients prior to and around conception may also affect maternal health and pregnancy outcomes. Periconceptional folic acid supplementation protects against neural tube defects<sup>(15)</sup>. Physiological adaptive processes ensure that the higher Ca needs for fetal growth are met without requiring an increase in maternal intake<sup>(16)</sup>, but it is not known whether low Ca intake prior to conception, or during pregnancy, affects the long-term health of the mother and her offspring<sup>(16)</sup>.

Pregnancy is a period in which women are particularly concerned with their diet and health, and frequently change their habits even if only temporarily. There are no Portuguese nutritional guidelines for pregnant women, so health professionals base their recommendations on the Dietary

\*Corresponding author: Email ecbpinto@med.up.pt

Reference Intakes (DRI) developed for the US population<sup>(17,18)</sup>. The objectives of the present study were to assess dietary intake among Portuguese women prior to conception and during pregnancy, and to evaluate their adequacy.

## Subjects and methods

### Subjects

This study was nested within Geração XXI, a birth cohort assembled in Porto, Portugal. All mothers resident in the metropolitan area of Porto who delivered a live-born baby between 1 May 2005 and 31 August 2006 in the maternity clinics of the five public hospitals were invited to participate in Geração XXI. These hospitals are responsible for 91.6% of the deliveries in the whole catchment population, with the remaining occurring in private hospitals/clinics. A total of 8654 babies were enrolled into the study. The present study is based on a sub-sample of mothers in this cohort who were followed up throughout pregnancy. All pregnant women who attended their first antenatal visit at Júlio Dinis Maternity Hospital or S. João Hospital between 1 December 2004 and 31 December 2005 were invited to participate if they reported a gestational age below 13 weeks. The invitations were made consecutively until the final size of the sub-sample was reached. Those who agreed to participate were interviewed in each trimester of pregnancy and in the immediate postpartum period. A sample size of 250 women will allow precise estimation of the true prevalence of intake inadequacy (i.e. 95% confidence intervals around the point estimate with a width of  $\pm 5\%$  for true prevalence of  $\leq 20\%$  or  $\geq 80\%$ , and a maximum of  $\pm 7\%$  for true prevalence between 20% and 80%).

A total of 430 pregnant women were initially enrolled in the two participating hospitals (participation rate 96.2%), with 249 completing the whole study. Forty-eight were excluded because of incorrect reporting of gestational age (as assessed by ultrasound) and thirty-one due to miscarriages, fetal deaths, stillbirths or very preterm deliveries (gestational age  $< 32$  weeks). A further ninety-nine women were excluded because, for logistic reasons, they were unable to complete both of the two FFQ (described later) and a further three because they provided unreliable dietary data (i.e. total energy intake outside mean  $\pm 3$ SD). Although the 249 participating women were a subset of the 430 initially enrolled, the two groups had similar baseline characteristics: there were no differences between those who participated and all those who were initially enrolled in terms of their age (mean (SD): 28.9 (5.8) *v.* 29.2 (6.6) years, respectively;  $P = 0.54$ ), number of completed years of schooling (mean (SD): 9.2 (4.1) *v.* 9.4 (3.6) years, respectively;  $P = 0.39$ ) or marital status (86.3% *v.* 86.1% were married, respectively;  $P = 0.53$ ), but those who participated were more likely to be primiparous (62.7% *v.* 54.8%, respectively;  $P = 0.07$ ).

Ethical approval for the study was obtained from relevant institutional ethics committees. All participants provided written informed consent.

### Data collection

Data were collected in each trimester of pregnancy and in the postpartum by trained interviewers using a structured questionnaire to obtain information on demographic and lifestyle variables, past medical history, health status during pregnancy, and use of vitamin and mineral supplements (including details on type of supplements and timing of their initiation and cessation). Education was recorded as the number of completed years of schooling and categorized as  $\leq 6$ , 7–9, 10–12 and  $> 12$ . Women were asked to estimate their monthly household income in 500€ categories. Height and weight were measured at each follow-up visit. Pre-pregnancy BMI was estimated from self-reported pre-pregnancy weight or, if this was not known (14.5% of women), the weight measured at the first visit. BMI was analysed according to WHO categories (underweight:  $< 18.5$  kg/m<sup>2</sup>; normal weight: 18.5–24.9 kg/m<sup>2</sup>; overweight: 25.0–29.9 kg/m<sup>2</sup>; obesity:  $\geq 30.0$  kg/m<sup>2</sup>)<sup>(19)</sup>.

### Dietary and nutrient intake estimates

Dietary intake was assessed by a semi-quantitative FFQ, comprising eighty-six food or food group items. Frequency of consumption was recorded into nine pre-specified categories from 'never or less than once per month' to 'six or more times per day'. Pre-specified portion sizes were allocated to each food item. Dietary intake was estimated by multiplying the frequency of intake for any given item by its respective portion size, in grams, and by a seasonal variation factor for foods consumed only in some seasons. The FFQ had been specially developed and validated for use among the Portuguese adult population<sup>(20)</sup> and, more recently, for use among pregnant women to estimate their dietary intake during the whole pregnancy (paper in preparation). The reference method for the latter was the average of three 3 d food diaries (FD), one from each trimester of pregnancy, collected from a subset of 101 pregnant women who participated in the present study. The percentage of pregnant women classified into the same or an adjacent quintile by the two methods was 60% for total energy and 60%, 58% and 55% for protein, carbohydrates and total fat, respectively. The corresponding percentages for selected micronutrients were 60% for vitamin E, 66% for folate, 69% for Mg and 73% for Fe. Extreme disagreement between the two methods (i.e. classification into opposite quintiles) was  $\leq 5\%$  for all nutrients. Bland–Altman plots showed no evidence of biases.

The FFQ was administered twice. The first administration (FFQ1) occurred at the time of the first antenatal visit in the first trimester of pregnancy, and aimed to estimate usual dietary intake in the year preceding the current pregnancy. The second FFQ (FFQ2) was administered a

few days after delivery to estimate usual dietary intake during the whole pregnancy.

The Food Processor Plus<sup>®</sup> program version SQL (ESHA Research, Salem, OR, USA) was used to convert food intakes into nutrient intakes. This database was supplemented with the nutritional composition of Portuguese foods and recipes, using data from Portuguese food composition databases<sup>(21,22)</sup> and national<sup>(23–26)</sup> and international<sup>(27–30)</sup> publications. If the nutrient composition of a dish was unknown, this was estimated on the basis of its culinary recipe and the nutrient composition of its ingredients.

Estimated Energy Requirements (EER) were calculated using the equation<sup>(31)</sup>:  $EER = 354 - 6.9 \times \text{age (years)} + \text{PAL}[9.36 \times \text{weight (kg)} + 726 \times \text{height (m)}]$ , with physical activity level (PAL) assumed to be low active (PAL = 1.12) as this is compatible with the activity level of the majority of Portuguese women<sup>(32)</sup>. The average EER for the whole pregnancy was calculated by adding a further 1185 kJ (283 kcal) to the previous calculation, as daily energy requirements increase, on average, by 0 kJ (0 kcal) in the first trimester, 1465 kJ (350 kcal) in the second and 2093 kJ (500 kcal) in the third (daily mean for the whole pregnancy = 1185 kJ (283 kcal))<sup>(33)</sup>. Inadequacy in nutrient intake was calculated by taking as cut-off points the Acceptable Macronutrient Distribution Ranges (AMDR) for macronutrients and the Estimated Average Requirements (EAR) for micronutrients, according to recommendations for the DRI of the US population<sup>(17,18,34,35)</sup>. Inadequacies of Ca and vitamin D intakes were estimated on the basis of Adequate Intakes (AI) as there is no EAR for these micronutrients. Recommended levels were defined as those appropriate for non-pregnant and pregnant women aged 19–50 years. Prevalence of intake inadequacy was estimated as the percentage of participants whose intake was outside (for macronutrients) or below (for micronutrients) recommended levels.

### Statistical analysis

The Kolmogorov–Smirnov test was used to assess the assumption of normality. Only total energy intake had a normal distribution and, hence, the paired *t* test was used to compare mean differences in its intake between the two time periods. For all other nutrients and foods, the Wilcoxon signed-rank test was used to compare median differences in intake between paired observations. Prevalences in the inadequacy of intake of specific nutrients (with 95% confidence intervals) were estimated, and logistic regression analyses conducted to identify potential socio-economic and behavioural determinants. Likelihood ratio tests and Wald tests were used to examine heterogeneity and linear trend, respectively, in the prevalence of intake inadequacy<sup>(36)</sup>. All statistical analyses were performed with the Statistical Package for the Social Sciences version 14.0 (SPSS Inc., Chicago, IL, USA) and Epi Info<sup>™</sup> version 6.0 (Centers for Disease Control and Prevention, Atlanta, GA, USA) statistical software packages.

## Results

### Participants' characteristics

The baseline characteristics of the study subjects are shown in Table 1. Participants had a mean age of 29 (SD 5.8) years at entry into the study. The median number of completed years of schooling was 9 (interquartile range (IQR) 6, 12); 86.3% were married, 59.5% were in active employment and 43.5% had family monthly income below 1000€, a figure equivalent to 2.5 times the national minimum wage. Fifty-seven per cent of women were of normal weight prior to becoming pregnant. The pregnancy was unplanned for 43.4% of women, and it was the first delivery for 62.7%. Over half of participants experienced nausea and/or vomiting in the first trimester, but this percentage decreased with increasing gestational length. A quarter of the women reported smoking in the first trimester of pregnancy, but this percentage fell to 15.3% and 13.4% in the second and third trimesters,

**Table 1** Characteristics of the study participants: sub-sample (*n* 249) of mothers in the Geração XXI birth cohort, Porto, Portugal, December 2004 to December 2005

	<i>n</i> †	%
Maternal age (years) at entry into the study		
≤20	29	11.6
21–30	124	49.8
31–40	96	38.6
Maternal education (years of schooling)		
≤6	79	31.7
7–9	73	29.3
10–12	65	26.1
>12	32	12.9
Current marital status		
Married	215	86.3
Not married	34	13.7
Current employment status		
Student	49	19.8
Employed	147	59.5
Unemployed	51	20.7
Current aggregate family income (€/month)		
<500	16	7.4
500–1000	78	36.1
1001–1500	80	37.0
>1500	42	19.4
Maternal pre-pregnancy BMI (kg/m <sup>2</sup> )		
<18.5	8	3.4
18.5–24.9	136	57.4
25.0–29.9	68	28.4
≥30.0	25	10.5
Parity		
0	156	62.7
≥1	93	37.3
Assisted reproduction	13	5.2
Planned pregnancy	141	56.6
Nausea and/or vomiting during pregnancy		
1st trimester	133	54.5
2nd trimester	71	33.3
3rd trimester	42	19.6
Smoking during pregnancy		
1st trimester	62	25.0
2nd trimester	38	15.3
3rd trimester	33	13.4
Use of illicit drugs during pregnancy	8	3.2

†Totals do not always add up to 249 due to missing values.

respectively. Only 3.2% reported to have ever used illicit drugs during pregnancy.

### Food consumption

Table 2 shows usual daily food consumption prior to and during pregnancy. Average daily consumption of milk and dairy products during pregnancy was almost twice that in the preceding year. Intake of fats, bread, fruits and soup also increased significantly with pregnancy whereas intake of eggs, red meat, rice, pasta and potatoes, fast food, alcoholic beverages, and coffee and tea decreased significantly. There were no statistically significant differences in the consumption of fish, vegetables, sweets, soft drinks and fruit juices between the two periods. The daily number of meals increased throughout pregnancy, with the percentage of women who had more than 5 meals/d rising from 54.3% in the first trimester to 71.5% in the third ( $P$  for linear trend  $<0.001$ ).

### Nutrient intakes and their adequacy

The daily intake of total energy during pregnancy was not statistically significantly different from that in the preceding year, but the percentage of energy derived from protein and saturated fat increased slightly with pregnancy (Table 3). Pregnancy was also accompanied by statistically significant increases in the intake of vitamins A and E, riboflavin, folate, Ca and Mg (Table 3). In contrast, the percentage of women who reported ever drinking alcoholic beverages fell from 36.3% prior to pregnancy to only 13.3% during it, with the median intake among drinkers declining from 3.7 g to 0.9 g between the two time periods. Almost all women consumed caffeine prior to and during pregnancy, but the median daily intake was reduced from 65 mg to 34 mg between the two periods (Table 3).

Daily mean energy intake was higher than EER both prior to (10 157 kJ (2426 kcal) *v.* 8252 kJ (1971 kcal), respectively;  $P < 0.001$ ) and during pregnancy (10 429 kJ (2491 kcal) *v.* 9437 kJ (2254 kcal), respectively;  $P < 0.001$ ). However, the mean difference between reported and recommended levels was much higher prior to pregnancy than during it (1905 kJ (455 kcal) *v.* 992 kJ (237 kcal), respectively). The reported intakes of total fat and carbohydrates prior to pregnancy were outside the AMDR for respectively 20.6% and 13.7% of the participating women, reflecting mainly under-consumption of carbohydrates and over-consumption of total fat. These levels of inadequacy were little affected by pregnancy.

Overall, the prevalence of micronutrient inadequacy was higher during pregnancy than prior to it. The nutrients with higher inadequacy prevalences prior to pregnancy were vitamin E (83.1%), folate (58.2%) and Mg (18.5%; Table 3). These three nutrients, together with Fe, were also those with the highest inadequacy prevalences during pregnancy (90.8% for folate, 88.0% for Fe, 73.1% for vitamin E and 21.3% for Mg; Table 3). As average levels of Ca intake were above recommendations in both periods, it is likely that the prevalence of inadequacy for this nutrient was rather low. In contrast, the intake of vitamin D was below recommended levels in both periods, but this inadequacy is of little concern to the Portuguese population given its high levels of exposure to sunlight.

The FFQ2 estimates of prevalence of inadequacy for the subset of 101 subjects who also completed a 3 d FD in each trimester of pregnancy were similar to those found for the whole sample of 249 women (Tables 3 and 4). The estimates of prevalence of inadequacy provided by the average of the three FD were similar to those provided by FFQ2 in this subset of women, except that the FD yielded

**Table 2** Median daily consumption of foods (in grams) among the study population in the year preceding pregnancy and during pregnancy: sub-sample ( $n$  249) of mothers in the Geração XXI birth cohort, Porto, Portugal, December 2004 to December 2005

Food group†	Preconceptional food intake (FFQ1)		Food intake during pregnancy (FFQ2)		Difference		$P$ ‡
	Median	IQR	Median	IQR	Median	IQR	
Milk and dairy products	387.5	267.0, 735.0	691.8	384.7, 923.5	196.1	0.0, 423.4	$<0.001$
Eggs	22.2	7.4, 22.2	11.1	7.4, 22.2	0.0	-7.4, 0.0	0.014
White meat	51.4	25.7, 68.6	51.4	25.1, 59.4	0.0	-25.7, 8.3	0.016
Red meat	68.0	55.9, 84.6	62.4	51.4, 74.1	-4.7	-31.0, 9.9	$<0.001$
Fish	60.2	38.1, 94.4	65.1	40.8, 98.0	0.0	-21.9, 27.9	0.544
Fats	12.7	8.2, 17.6	13.6	7.8, 21.5	0.0	0.0, 0.0	0.032
Bread	123.3	91.0, 157.6	131.4	92.3, 179.7	8.1	-42.9, 64.2	0.046
Rice, pasta, potatoes	172.1	132.9, 214.3	161.4	118.6, 199.1	-4.3	-62.3, 35.7	0.047
Sweets	65.2	37.8, 97.0	54.3	29.5, 93.1	-4.3	-39.6, 27.5	0.121
Vegetables	126.5	76.9, 188.2	124.8	69.5, 221.1	4.2	-52.0, 66.2	0.325
Fruits	313.3	226.5, 434.5	336.2	233.0, 498.9	21.5	-83.4, 159.8	0.029
Fast food	25.1	8.0, 48.1	17.1	8.0, 38.4	-3.3	-20.6, 9.1	0.001
Soup	231.8	65.8, 295.0	295.0	126.4, 295.0	0.0	0.0, 168.6	$<0.001$
Alcoholic beverages	0.0	0.0, 22.0	0.0	0.0, 0.0	0.0	-17.9, 0.0	$<0.001$
Coffee and tea	101.6	19.3, 128.3	45.0	0.0, 60.8	-15.8	-101.6, 0.0	$<0.001$
Soft drinks and fruit juices	258.0	106.6, 519.2	131.5	29.7, 285.5	-85.7	-206.7, 43.7	0.122

IQR, interquartile range.

†Food groups as defined by Erkkola *et al.*<sup>(39)</sup>.

‡ $P$  values calculated using the Wilcoxon signed-rank test.

**Table 3** Recommended dietary reference intakes (DRI), usual dietary daily nutrient intake and prevalence of intake inadequacy prior to and during pregnancy among the study subjects: sub-sample (*n* 249) of mothers in the Geração XXI birth cohort, Porto, Portugal, December 2004 to December 2005

Nutrient	Year preceding pregnancy					During pregnancy				
	Usual daily dietary intake (FFQ1)					Usual daily dietary intake (FFQ2)				
	Prevalence of inadequacy					Prevalence of inadequacy				
	DRI	Median	IQR	%	95% CI	DRI	Median	IQR	%	95% CI
Energy (kJ)		10019	8261, 11706				10145	8629, 11882		
Energy (kcal)		2393	1973, 2796				2423	2061, 2838		
Carbohydrates (% of energy)	45–65†	49.5	42.5, 60.0	13.7	9.4, 17.9	45–65†	50.3	42.3, 62.1	10.8	7.0, 14.7
Total fat (% of energy)	20–35†	31.0	25.6, 37.3	20.6	15.5, 25.5	20–35†	30.6	26.1, 39.0	21.0	15.8, 25.9
SFA (% of energy)		10.0	8.1, 12.7				10.5	8.7, 13.1		
MUFA (% of energy)		13.2	11.0, 15.9				13.0	10.6, 16.9		
PUFA (% of energy)		4.8	4.0, 6.0				4.6	3.8, 6.1		
Protein (% of energy)	10–35†	17.6	14.6, 20.7	0.0	0.0, 1.5	10–35†	18.4	15.4, 21.1	0.0	0.0, 1.5
Vitamin A (µg)	500.0§	1583.9	1135.9, 2289.4	5.6	2.8, 8.5	550.0§	1711.1	1162.2, 2512.6	3.6	1.3, 5.9
Vitamin C (mg)	60.0§	138.7	105.7, 176.7	3.2	1.0, 5.4	70.0§	142.6	110.7, 195.7	8.8	5.3, 12.4
Vitamin D (µg)	5.0	3.9	2.8, 5.4			5.0	3.6	2.7, 5.5		
Vitamin E (mg)	12.0§	8.9	7.2, 11.0	83.1	78.0, 87.4	12.0§	9.3	7.5, 12.3	73.1	67.6, 78.6
Thiamin (mg)	0.9§	1.9	1.6, 2.2	0.0	0.0, 1.5	1.2§	1.9	1.6, 2.3	5.6	2.8, 8.5
Riboflavin (mg)	0.9§	2.4	1.9, 2.9	0.0	0.0, 1.5	1.2§	2.7	2.2, 3.3	3.2	1.0, 5.4
Niacin (mg)	11.0§	24.6	20.3, 28.3	0.4	–0.38, 1.2	14.0§	23.4	19.0, 28.0	5.2	2.5, 8.0
Pyridoxine (mg)	1.1§	2.3	1.9, 2.7	0.8	–0.31, 1.9	1.6§	2.3	2.0, 2.8	9.6	6.0, 13.3
Folate (µg)	320.0§	293.5	239.4, 380.1	58.2	52.1, 64.4	520§	314.6	245.1, 400.8	90.8	87.2, 94.4
Vitamin B <sub>12</sub> (µg)	2.0§	9.1	6.4, 12.1	0.0	0.0, 1.5	2.2§	9.0	6.6, 12.3	0.0	0.0, 1.5
Ca (mg)	1000.0	1067.8	799.6, 1439.2			1000.0	1380.1	1060.6, 1750.5		
Mg (mg)	255.0§	331.5	271.3, 400.9	18.5	13.7, 23.3	290.0§	362.4	301.2, 429.6	21.3	16.2, 26.4
Fe (mg)	8.1§	15.9	13.3, 18.8	0.0	0.0, 1.5	22.0§	16.0	12.5, 19.2	88.0	83.9, 92.0
Alcohol (g)		0.0	0.0, 1.6				0.0	0.0, 0.0		
Caffeine (mg)		64.8	31.8, 99.7				34.4	17.3, 60.4		

FFQ1, FFQ on usual diet prior to pregnancy; FFQ2, FFQ on usual diet during the whole pregnancy; IQR, interquartile range.

†P value for the comparison of average nutrient intake between the two time periods.

§Acceptable Macronutrient Distribution Range.

||Estimated Average Requirement.

||Adequate Intake.



significantly higher estimates for vitamins C and E, pyridoxine and Mg than the FFQ2 (Table 4).

Overall, there were no clear relationships between the socio-economic and behavioural characteristics and the prevalence of nutritional inadequacy during pregnancy. After adjustment for maternal age and education, inadequacy in vitamin E intake was higher if the pregnancy had been planned (OR = 2.13; 95% CI 1.18, 3.87;  $P = 0.01$ ); inadequacy in Fe intake was higher among women who were overweight ( $BMI \geq 25 \text{ kg/m}^2$ ) prior to becoming pregnant (OR = 3.45; 95% CI 1.22, 9.71;  $P = 0.01$ ), but lower among those with higher educational level ( $P$  for linear trend = 0.01) and those who reported suffering

from nausea and/or vomiting in the first trimester (OR = 0.38; 95% CI 0.16, 0.91;  $P = 0.03$ ).

### Vitamin and mineral supplements

Data on use of vitamins and minerals supplements were available for 99% of the participating women (Table 5). Only 18.6% reported preconception supplements of folic acid, but 96.8% reported taking them during the first trimester and 73.5% during the third. The median gestational age at initiation of folic acid supplementation was 6.5 (IQR 5, 9) weeks. The self-reported prevalence of Fe supplementation increased from 42.2% in the first trimester to 75.9% in the third, with a median gestational

**Table 4** Prevalence of inadequacy in nutrient intake during pregnancy as estimated by the FFQ completed in the puerperium (FFQ2) and the average of three 3 d food diaries (FD) in the subset of women ( $n$  101) who complied with both dietary methods: Geração XXI birth cohort, Porto, Portugal, December 2004 to December 2005

Nutrient	DRI	Prevalence of inadequacy during the whole pregnancy as estimated by			
		FFQ2		Average of three 3 d FD†	
		%	95% CI	%	95% CI
Protein (% of energy)	10–35‡	0.0	0.0, 2.9	0.0	0.0, 2.9
Carbohydrates (% of energy)	45–65‡	11.9	6.6, 19.3	16.8	10.4, 25.1
Total fat (% of energy)	20–35‡	25.7	17.9, 34.9	35.6	26.8, 45.3
Vitamin A ( $\mu\text{g}$ )	550.0§	4.0	1.3, 9.3	9.9	5.1, 16.9
Vitamin C (mg)	70.0§	8.9	4.4, 15.7	26.7*	18.8, 36.0
Vitamin E (mg)	12.0§	72.3	63.0, 80.3	89.1*	81.9, 94.1
Thiamin (mg)	1.2§	3.0	0.8, 7.9	2.0	0.3, 6.4
Riboflavin (mg)	1.2§	1.0	0.0, 4.8	2.0	0.3, 6.4
Niacin (mg)	14.0§	3.0	0.8, 7.9	1.0	0.0, 4.8
Pyridoxine (mg)	1.6§	7.9	3.7, 14.5	20.8*	13.7, 29.5
Folate ( $\mu\text{g}$ )	520.0§	92.1	85.5, 96.3	90.1	83.1, 94.9
Vitamin B <sub>12</sub> ( $\mu\text{g}$ )	2.2§	0.0	0.0, 2.9	0.0	0.0, 2.9
Mg (mg)	290.0§	15.8	9.7, 23.9	46.5*	37.0, 56.3
Fe (mg)	22.0§	88.1	80.7, 93.4	93.1	86.8, 96.9

\*Statistically significantly different ( $P < 0.05$ ) from the corresponding FFQ2 estimate in this subset of women.

†One 3 d FD completed in each trimester of pregnancy.

‡Acceptable Macronutrient Distribution Range.

§Estimated Average Requirement.

**Table 5** Prevalence of intake of selected vitamin and mineral supplements prior to and during pregnancy among the study subjects: sub-sample ( $n$  249) of mothers in the Geração XXI birth cohort, Porto, Portugal, December 2004 to December 2005†

Timing of supplementation	Supplementation of		
	Folic acid	Fe	Mg
Preconception			
%	18.6	1.6	0.0
95% CI	14.1, 23.8	0.5, 3.9	0.0, 2.0
Gestational age at initiation (weeks)			
Median	6.5	16.0	24.0
IQR	5.0, 9.0	11.0, 20.0	14.0, 30.0
First trimester			
%	96.8	42.2	12.0
95% CI	94.0, 98.5	36.1, 48.4	8.4, 16.5
Second trimester			
%	90.0	76.7	28.5
95% CI	85.0, 93.2	71.2, 81.6	23.2, 34.4
Third trimester			
%	73.5	75.9	36.1
95% CI	67.7, 78.7	70.3, 80.9	30.4, 42.3

IQR, interquartile range.

†Data missing for two (0.8%) women.

age at initiation of 16 (IQR 11, 20) weeks. Mg supplements were taken by 12.0% of the women in the first trimester and by 36.1% in the third, with a median gestational age at initiation of 24 (IQR 14, 30) weeks.

## Discussion

### Main findings

The present study showed that the intakes of energy and macronutrients were within recommended levels for most pregnant women. Energy requirements increase with pregnancy. In our sample there was no increase in energy intake with pregnancy but, despite this, energy intake remained above recommended levels. Thus, and in contrast to English pregnant women<sup>(37)</sup>, we did not find any energetic deficit during gestation. As important as total energy intake is its distribution by the different macronutrients. The observed inadequacy in carbohydrates intake prior to and during pregnancy was mainly a result of low consumption, whereas the inadequacy in fat intake reflected mainly overconsumption. Protein intake was adequate in both time periods. These findings are in line with the dietary habits of the Portuguese adult population<sup>(38)</sup>.

Preconceptional dietary intakes of vitamin E, folate and Mg were below the recommended levels for large proportions of women. The average daily intakes of these micronutrients increased with pregnancy, but as their requirements also increased, the prevalences of inadequacy in the intake of folate and Mg, although not of vitamin E, were higher during pregnancy than in the preceding year. The prevalence of inadequacy in vitamin E intake may have been, at least in part, overestimated as both the FFQ and FD may have underestimated the amount of fats added to dishes after cooking. The level of inadequacy in folate intake reported here is similar to that observed among Finnish pregnant women<sup>(39)</sup>. A recent US study<sup>(40)</sup> reported a much lower inadequacy prevalence (36% *v.* 90.8% in our sample) but, in contrast to Portugal, folate fortification of staple foods is common in the USA. A much lower prevalence was also observed in a large British study but the cut-off points used to define inadequacy of folate intake were very different<sup>(37)</sup> from those used in the present study. Intake of Fe prior to conception was within recommended levels for most women, but as requirements almost triple during pregnancy with no parallel increase in intake, the prevalence of inadequacy was very high during pregnancy. Average levels of intake of vitamin C, known to increase absorption of Fe, were well above recommended levels in both time periods.

The intakes of most macro- and micronutrients in our study were higher than those reported for pregnant women in England<sup>(37,41)</sup>, but similar to those found among pregnant women in Finland<sup>(39)</sup>, the USA<sup>(42)</sup> and Greece<sup>(43)</sup>.

We attempted to identify groups of women who may be at particular risk of nutrient inadequacy and to whom dietary interventions should be targeted. We found no clear associations between the social and behavioural characteristics of the participants and intake inadequacy. These findings should be interpreted with caution as the power of the study to detect these associations was low. Conversely, the few observed associations may have arisen by chance given the large number of statistical tests performed.

Randomised trials<sup>(44–48)</sup> have shown that folic acid supplementation during the periconceptional period and early pregnancy can reduce the risk of neural tube defects by 80% or more. Practically all women in the present study took folic acid supplements. However, few took them during the most critical period<sup>(49)</sup>. In our sample only 18.6% of women used folic acid in the three months prior to becoming pregnant and only 20.2% initiated supplementation before the fourth week of gestation. These values probably underestimate the true prevalence of folate inadequacy in the general population as our sample excluded pregnant women who had their first routine antenatal visit after 12 weeks of gestation and were thus less likely to have taken folic acid supplements in early pregnancy.

Mg supplementation in the first trimester was uncommon but it became more frequent subsequently. The main reasons for prescribing this supplement are abdominal pains and muscular cramps, not concerns of a possible nutritional deficiency. Thus, in the absence of symptoms, there is probably no justification to treat Mg deficiencies.

### Strengths and weaknesses

Our study has some strengths. First, it used a paired design to compare food and nutrient intake prior to and during pregnancy in the same sample of women, thus minimising the potential for confounding by maternal characteristics. Second, a subset of 101 women was able to complete the various FFQ as well as a 3d FD in each trimester of pregnancy. The study has also some weaknesses. Our sample was assembled in two public hospitals in Porto and therefore any extrapolations to the general population should be made with caution. Comparison with national statistics showed that the age composition of our sample was similar to that for all Portuguese pregnant women who delivered a live-born baby in the same time period (mean (95% CI): 28.6 (28.1, 29.6) *v.* 29.6 years, respectively), but our participants were more likely to be primiparous (62.7% (56.3%, 68.7%) *v.* 54.4%, respectively), less likely to be married (86.3% (81.4%, 90.4%) *v.* 94.0%, respectively) and slightly less educated (percentage with <10 years of schooling: 60.9% (54.5%, 67.0%) *v.* 49.7%, respectively).

Because of the study design women with a gestational age above 13 weeks at entry and those whose pregnancies ended in miscarriages, stillbirths and very premature



births (gestational age <32 weeks) were excluded. As diet has been shown to be associated with adverse pregnancy outcomes<sup>(50)</sup>, these exclusions may have affected the estimates of food and nutrient intakes reported here.

The FFQ was chosen to assess dietary intake in our study as it allowed retrospective estimation of diet prior to and around conception. The FFQ is an appropriate tool for epidemiological studies where the main objective is to rank individuals according to their levels of intake and to identify extremes of intake<sup>(51–53)</sup>. Although an FFQ was used in the present study to quantify absolute intake, this approach seems reasonable because of the paired design. Comparison of the FFQ estimates with those derived from the average of the three 3 d FD in a subset of women show that, if anything, the FFQ underestimated somehow the prevalence of inadequacy in the intake of certain nutrients. This subset of women is unlikely to be unrepresentative as their FFQ estimates were similar to those for the whole study population. The differences between the two methods may partly reflect the fact that the FFQ tends to overestimate usual intake whereas the FD tends to underestimate it<sup>(53,54)</sup>.

Preconceptional diet was assessed retrospectively in early pregnancy and the interviewers were instructed to remind women of the exact reference period several times throughout the FFQ administration. Recall bias due to pregnancy complications can be eliminated because the FFQ was administered in early pregnancy, but recall bias due to social desirability and nausea/vomiting cannot be excluded. Estimates of the usual dietary intake throughout the whole pregnancy were also obtained retrospectively, a few days after delivery. Knowledge of the pregnancy outcome could have affected recall of diet by the participants, but it is unlikely that this might have biased our findings considerably because the inadequacy prevalence estimates based on FFQ2 were similar to those derived from the FD in the subset of women who completed these prospectively throughout pregnancy.

### Public health implications

Although nutritional requirements increase with pregnancy<sup>(34,35)</sup> as a result of maternal physiological changes and progressive fetal growth and development<sup>(55)</sup>, few dietary changes are required within well-nourished populations with a balanced diet<sup>(56)</sup>. The present study showed that the diet of Portuguese women prior to conception and during pregnancy is likely to contain adequate amounts of most nutrients, except vitamin E, folate and Mg. Fe intake during pregnancy was also below recommended levels.

The prevalence of inadequacy in dietary Fe intake was high in our study. The physiological requirements of Fe tend to increase from mid-pregnancy<sup>(57)</sup>, at a time when over 75 % of pregnant women in the present study were on Fe supplementation. Moreover, Fe inadequacy can be easily detected by monitoring Hb levels throughout pregnancy.

Folic acid supplementation was widespread, but the timing of its initiation was inappropriate for most women. Portuguese national guidelines on folic acid, issued in 1998, recommended supplementation for all women of childbearing age<sup>(58)</sup>. Our findings of a low dietary intake of folate combined with very late initiation of supplementation, nine years after the introduction of the current national guidelines, are disappointing. A recent analysis of data from ten European countries, including Portugal, showed that the issuing of recommendations on folate dietary intake and supplementation was not followed by a decline in the prevalence of neural tube defects in any of the populations studied<sup>(59)</sup>. In contrast, the introduction of population-based measures such as the widespread fortification of staple foods with folate was an efficient and low-cost approach in the USA, Canada, Chile and South Africa<sup>(60–63)</sup>. The introduction of widespread folate fortification in Portugal should be considered as a possible complementary approach to the current recommendations on dietary intake and supplements.

### Acknowledgements

*Sources of funding:* The study was funded by Programa Operacional de Saúde – Saúde XXI, Quadro Comunitário de Apoio III and by Administração Regional de Saúde Norte. E.P. was funded by Fundação para a Ciência e a Tecnologia (SFRH/BD/19803/2004).

*Conflict of interest declaration:* There are no conflicts of interest.

*Authors' contributions:* H.B. and I.S.S. were responsible for designing the study and for obtaining funding. E.P. was responsible for subject recruitment, data collection, and all of the nutritional and statistical analyses. E.P. wrote the manuscript jointly with H.B. and I.S.S. All three authors contributed to the interpretation of the findings and the final version of the manuscript.

*Acknowledgement:* We are grateful to the families enrolled in the Geração XXI study for their generosity; the various members of the research team for their enthusiasm and perseverance; and the participating hospitals and their staff for their help and support. In particular, we would like to thank P. Sarmiento, A.C. Cunha, A. Gouveia, E. Fernandes and L. Fernandes from Júlio Dinis Maternity Hospital, and N. Montenegro, M. Moucho, T. Rodrigues and L. Francisco from S. João Hospital.

### References

1. Moore V, Davies M, Willson K, Worsley A & Robinson JS (2004) Dietary composition of pregnant women is related to size of the baby at birth. *J Nutr* **134**, 1820–1826.
2. Harding J (2001) The nutritional basis of the fetal origins of adult disease. *Int J Epidemiol* **30**, 15–23.
3. Kind K, Moore V & Davies M (2006) Diet around conception and during pregnancy – effects on fetal and neonatal outcomes. *Reprod Biomed Online* **12**, 532–541.

4. Olafsdottir A, Skuladottir G, Thorsdottir I, Hauksson A, Thorgeirsdottir H & Steingrimsdottir L (2006) Relationship between high consumption of marine fatty acids in early pregnancy and hypertensive disorders in pregnancy. *BJOG* **113**, 301–309.
5. Moore V & Davies M (2005) Diet during pregnancy, neonatal outcomes and later health. *Reprod Fertil Dev* **17**, 341–348.
6. Fowles E (2004) Prenatal nutrition and birth outcomes. *J Obstet Gynecol Neonatal Nurs* **33**, 809–822.
7. Luke B (2005) The evidence linking maternal nutrition and prematurity. *J Perinat Med* **33**, 500–505.
8. Godfrey K, Robinson S, Barker D, Osmond C & Cox V (1996) Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. *BMJ* **312**, 410–414.
9. Olsen S, Sorensen J, Secher N, Hedegaard M, Henriksen T, Hansen H & Grant A (1992) Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. *Lancet* **339**, 1003–1007.
10. Worthington-Roberts B (1997) The role of maternal nutrition in the prevention of birth defects. *J Am Diet Assoc* **97**, S184–S185.
11. Barker D (1998) *Mothers, Babies and Health in Later Life*. Edinburgh: Churchill Livingstone.
12. Frederick I, Williams M, Dashow E, Kestin M, Zhang C & Leisenring W (2005) Dietary fiber, potassium, magnesium and calcium in relation to the risk of preeclampsia. *J Reprod Med* **50**, 332–344.
13. Siega-Riz A, Evenson K & Dole N (2004) Pregnancy-related weight gain – a link to obesity? *Nutr Rev* **62**, S105–S111.
14. Teles T, Rodrigues T & Barros H (1994) [Maternal anthropometric characteristics. Risk of intrauterine growth retardation]. *Acta Med Port* **7**, 669–675.
15. Lumley J, Watson L, Watson M & Bower C (2001) Periconceptional supplementation with folate and/or multivitamins for preventing neural tube defects. *Cochrane Database Syst Rev* issue 3, CD001056.
16. Prentice A (2000) Calcium in pregnancy and lactation. *Annu Rev Nutr* **20**, 249–272.
17. Food and Nutrition Board, Institute of Medicine (2001) *Dietary Reference Intakes*. Washington, DC: National Academy Press; available at <http://www.nap.edu>
18. Trumbo P, Schlicker S, Yates A & Poos M (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J Am Diet Assoc* **102**, 1621–1630.
19. World Health Organization (1995) *Physical Status: The Use and Interpretation of Anthropometry. Report of a WHO Expert Committee. WHO Technical Report Series* no. 854. Geneva: WHO.
20. Lopes C, Aro A, Azevedo A, Ramos E & Barros H (2007) Intake and adipose tissue composition of fatty acids and risk of myocardial infarction in a male Portuguese community sample. *J Am Diet Assoc* **107**, 276–286.
21. Ferreira F & Graça M (1985) *Tabela de composição de alimentos portugueses*, 2<sup>a</sup> ed. Lisbon: Instituto Nacional da Saúde Dr. Ricardo Jorge.
22. Centro de Segurança Alimentar e Nutrição (2006) *Tabela da Composição de Alimentos*. Lisbon: Instituto Nacional da Saúde Dr. Ricardo Jorge.
23. Amaral C, Sequeira C & Camacho M (1993) Iogurte – composição e valor nutritivo de variedades comercializadas em Portugal. Subsídio para a tabela de composição dos alimentos portugueses. *Rev Port Nutr* issue 3, 35–52.
24. Batista I & Bandarra N (1993) Influência de quatro métodos culinários na composição química de várias espécies de peixe. *Rev Port Nutr* issue 3, 5–14.
25. Mano M, Meister M, Fontes M & Lobo P (1989) Composição de alguns alimentos cozinhados. Alguns produtos servidos em 'snack-bares'. *Rev Port Nutr* issue 4, 19–24.
26. Mano M, Meister M, Fontes M & Lobo P (1989) Composição de sobremesas doces. *Rev Port Nutr* issue 1, 16–24.
27. Aro A, Amaral E, Kesteloot H, Rimestad A, Thamm M & van Poppel G (1998) *Trans* fatty acids in French fries, soups, and snacks from 14 European countries: the Transfair study. *J Food Compos Anal* **11**, 170–177.
28. Aro A, Antoine J, Pizzoferrato L, Reykdal O & Van Poppel G (1998) *Trans* fatty acids in dairy and meat products from 14 European countries: the Transfair study. *J Food Compos Anal* **11**, 150–160.
29. Aro A, Van Amelsvoort J, Becker W, Van Erp-baart M, Kafatos A, Leth T & van Poppel G (1998) *Trans* fatty acids in dietary fats and oils from 14 European countries: the Transfair study. *J Food Compos Anal* **11**, 137–149.
30. Van Erp-baart M, Couet C, Cuadrado C, Kafatos A, Stanley J & van Poppel G (1998) *Trans* fatty acids in bakery products from 14 European countries: the Transfair study. *J Food Compos Anal* **11**, 161–169.
31. Food and Nutrition Board, Institute of Medicine (2002) *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. Washington, DC: National Academy Press.
32. Gal D, Santos A & Barros H (2005) Leisure-time versus full-day energy expenditure: a cross-sectional study of sedentarism in a Portuguese urban population. *BMC Public Health* **5**, 16.
33. Butte N, Wong W, Treuth M, Ellis K & O'Brian Smith E (2004) Energy requirements during pregnancy based on total energy expenditure and energy deposition. *Am J Clin Nutr* **79**, 1078–1087.
34. Lachance P (1998) International perspective: basis, need, and application of recommended dietary allowances. *Nutr Rev* **56**, S2–S4.
35. Yates A (1998) Process and development of dietary reference intakes: basis, need, and application of recommended dietary allowances. *Nutr Rev* **56**, S5–S9.
36. Clayton D & Hills M (1993) *Statistical Models in Epidemiology*. Oxford: Oxford University Press.
37. Mouratidou T, Ford F, Prountzou F & Fraser R (2006) Dietary assessment of a population of pregnant women in Sheffield, UK. *Br J Nutr* **96**, 929–935.
38. Lopes C, Oliveira A, Santos AC, Ramos E, Severo M & Barros H (2006) Consumo Alimentar no Porto. [http://higiene.med.up.pt/consumoalimentarporto/download/re\\_cap\\_21062006.pdf](http://higiene.med.up.pt/consumoalimentarporto/download/re_cap_21062006.pdf) (accessed April 2007).
39. Erkkola M, Karppinen M, Jarvinen A, Knip M & Virtanen S (1998) Folate, vitamin D, and iron intakes are low among pregnant Finnish women. *Eur J Clin Nutr* **52**, 742–748.
40. Sherwood K, Houghton L, Tarasuk V & O'Connor D (2006) One-third of pregnant and lactating women may not be meeting their folate requirements from diet alone based on mandated levels of folic acid fortification. *J Nutr* **136**, 2820–2826.
41. Rogers I & Emmett P (1998) Diet during pregnancy in a population of pregnant women in South West England. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. *Eur J Clin Nutr* **52**, 246–250.
42. Wei E, Gardner J, Field A, Rosner B, Colditz G & Saito C (1999) Validity of a food frequency questionnaire in assessing nutrient intakes of low-income pregnant women. *Matern Child Health J* **3**, 241–246.
43. Petrakos G, Panagopoulos P, Koutras I, Kazis A, Panagiotakos D, Economou A, Kanellopoulos N, Salamalekis E & Zabelas A (2006) A comparison of the dietary and total intake of micronutrients in a group of pregnant Greek women with the Dietary Reference Intakes. *Eur J Obstet Gynecol Reprod Biol* **127**, 166–171.
44. Wald N, Sneddon J, Densem J, Frost C & Stone R (1991) Prevention of neural tube defects: results of the Medical

- Research Council Vitamin Study. MRC Vitamin Study Research Group. *Lancet* **338**, 131–137.
45. Kirke P, Daly L & Elwood J (1992) A randomised trial of low dose folic acid to prevent neural tube defects. The Irish Vitamin Study Group. *Arch Dis Child* **67**, 1442–1446.
  46. Czeizel A, Dudas I & Metneki J (1994) Pregnancy outcomes in a randomised controlled trial of periconceptional multivitamin supplementation. Final report. *Arch Gynecol Obstet* **255**, 131–139.
  47. Czeizel A, Dobo M & Vargha P (2004) Hungarian cohort-controlled trial of periconceptional multivitamin supplementation shows a reduction in certain congenital abnormalities. *Birth Defects Res A Clin Mol Teratol* **70**, 853–861.
  48. Central Technical Co-ordinating Unit, ICMR (2000) Multi-centric study of efficacy of periconceptional folic acid containing vitamin supplementation in prevention of open neural tube defects from India. *Indian J Med Res* **112**, 206–211.
  49. Goldberg B, Alvarado S, Chavez C, Chen B, Dick L, Felix R, Kao K & Chambers C (2006) Prevalence of periconceptional folic acid use and perceived barriers to the postgestation continuance of supplemental folic acid: survey results from a Teratogen Information Service. *Birth Defects Res A Clin Mol Teratol* **76**, 193–199.
  50. Maconochie N, Doyle P, Prior S & Simmons R (2007) Risk factors for first trimester miscarriage – results from a UK-population-based case-control study. *BJOG* **114**, 170–186.
  51. Erkkola M, Karppinen M, Javanainen J, Rasanen L, Knip M & Virtanen S (2001) Validity and reproducibility of a food frequency questionnaire for pregnant Finnish women. *Am J Epidemiol* **154**, 466–476.
  52. Torheim L, Barikmo I, Hatloy A, Diakite M, Solvoll K, Diarra M & Oshaug A (2001) Validation of a quantitative food-frequency questionnaire for use in Western Mali. *Public Health Nutr* **4**, 1267–1277.
  53. Willett W & Lenart E (1998) Reproducibility and validity of food-frequency questionnaires. In *Nutritional Epidemiology*, 2nd ed, pp 101–147 [WC Willett, editor]. New York: Oxford University Press.
  54. Cade J, Thompson R, Burley V & Warm D (2002) Development, validation and utilisation of food-frequency questionnaires – a review. *Public Health Nutr* **5**, 567–587.
  55. Brown J, Buzzard I, Jacobs D Jr, Hannan P, Kushi L, Barosso G & Schmid L (1996) A food frequency questionnaire can detect pregnancy-related changes in diet. *J Am Diet Assoc* **96**, 262–266.
  56. Anderson A (2001) Symposium on 'nutritional adaptation to pregnancy and lactation'. Pregnancy as a time for dietary change? *Proc Nutr Soc* **60**, 497–504.
  57. Bothwell T (2000) Iron requirements in pregnancy and strategies to meet them. *Am J Clin Nutr* **72**, 257S–264S.
  58. Directorate General of Health, Maternal, Child and Adolescence Division (2006) Preconceptional health care, 02/DSMIA, 16/01/06.
  59. Botto L, Lisi A, Robert-Gnansia E *et al.* (2005) International retrospective cohort study of neural tube defects in relation to folic acid recommendations: are the recommendations working? *BMJ* **330**, 571.
  60. Grosse S, Waitzman N, Romano P & Mulinare J (2005) Reevaluating the benefits of folic acid fortification in the United States: economic analysis, regulation, and public health. *Am J Public Health* **95**, 1917–1922.
  61. De Wals P, Tairou F, Van Allen M *et al.* (2007) Reduction in neural-tube defects after folic acid fortification in Canada. *N Engl J Med* **357**, 135–142.
  62. Llanos A, Hertrampf E, Cortes F, Pardo A, Grosse S & Uauy R (2007) Cost-effectiveness of a folic acid fortification program in Chile. *Health Policy* **83**, 295–303.
  63. Ingram C, Fleming A, Patel M & Galpin J (1999) Pregnancy- and lactation-related folate deficiency in South Africa – a case for folate food fortification. *S Afr Med J* **89**, 1279–1284.